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Osmotic control of prolactin release and its effect on renal water excretion in man

TOMAS BERL, NACHMAN BRAUTBAR, MENASHE BEN-DAVID, WALTER CZACZKES and CHARLES KLEEMAN

Department of Medicine, University of Colorado Medical School, Denver, Colorado and Departments of Nephrology, Pharmacology and Medicine, Hadassah Hebrew University, Jerusalem, Israel

Osmotic control of prolactin release and its effects on renal water excretion in man. Prolactin appears to play a role in osmoregulation of fishes and birds, and a possible contribution of this hormone to the regulation of salt and water excretion in mammals has been suggested as well. The present studies were undertaken to investigate the role of osmotic pressure on the secretion of prolactin and the effect of the hormone on renal water excretion in man. The i.v. administration of synthetic thyrotropin-releasing hormone (TRH) (7 μg/kg) to five subjects undergoing a maximal sustained water diuresis increased serum prolactin to supraphysiologic levels in all as mean concentration rose from 30.2 ± 2.9 to 60.2 ± 5.0 ng/ml ($P < 0.005$). This increase was not associated with either significant alterations in renal hemodynamics or sodium excretion and water excretion. The osmoregulation of prolactin release was then investigated by the oral administration of 20 ml/kg of water to seven subjects in 11 studies. While the water load decreased serum osmolality from 293 ± 1.7 to 285 ± 1.5 mOsm/kg H_2O ($P < 0.001$), there was no significant change in prolactin level, 28 ± 1.8 to 30 ± 2.4 ng/ml. Serum hypertonicity was achieved in six subjects with the infusion of 5% NaCl which increased serum osmolality from 287 ± 1.8 to 298 ± 1.4 mOsm/kg ($P < 0.001$). While the hypertonic state caused a marked antidiuresis as urinary osmolality rose from 62 ± 5.9 to 480 ± 48 mOsm/kg ($P < 0.001$), the concentration of prolactin remained unchanged at 28 ng/ml. We conclude that supraphysiologic levels of prolactin have no antidiuretic properties in a vasopressin-free state and that acute alterations in serum tonicity within the range observed do not affect the release of prolactin in man.

Contrôle osmotique de la libération de prolactine et effet de la prolactine sur l'excrétion rénale d'eau chez l'homme. La prolactine paraît jouer un rôle dans l'osmorégulation des poissons et des oiseaux et, de la même façon, une contribution de cette hormone à la régulation de l'excrétion d'eau et de sel chez les mammifères a été suggérée. Ce travail a été entrepris pour étudier le rôle de la pression osmotique sur la sécrétion de prolactine et l'effet de l'hormone sur l'excrétion rénale d'eau chez l'homme. L'administration intra-veineuse de thyrotropin releasing horome (Trh) de synthèse (7 μg/kg) à cinq sujets soumis à une diurèse aqueuse maimale a déterminé l'augmentation de la concentration sérique de prolactine à des niveaux supra-physiologiques (de 30,2 ± 2,9 à 60,2 ± 5,0 ng/ml, $P < 0,005$). Cette augmentation n'a été associée ni à une modification significative de l'hémodynamique rénale, ni à une modification significative de l'excrétion d'eau et de sodium. La régulation par l'osmolalité de la libération de prolactine a été explorée par l'administration d'eau (20 ml/kg) à sept sujets au cours de ll études. Alors que la charge en eau fait baisser l'osmolalité plasmatique de 293 ± 1,7 à 285 ± 1,5 mOsm/kg H_2O ($P < 0,001$) il n'y a pas de modification significative de la concentration de prolactine (28 ± 1,8 à 30 ± 2,4 ng/ml). Une hypertonicité a été réalisée chez six sujets par l'administration de NaCl 5%, ce qui a fait augmenter l'osmolalité de 287 ± 1,8 à 298 ± 1,4 mOsm/kg ($P < 0,001$). Alors que l'hypertonicité détermine une antidiurèse importante traduite par une augmentation de l'osmolalité urinaire de 62 ± 5,9 à 480 ± 48 mOsm/kg ($P < 0,001$), la concentration de prolactine est inchangée (28 ng/ml). Nous concluons que des concentrations supra-physiologiques de prolactine n'ont pas de propriétés antidiurétiques et que des modifications aiguës de l'osmolalité plasmatique, dans l'intervalle des valeurs observées, ne modifient pas la libération de prolactine chez l'homme.

The anterior pituitary hormone prolactin, which plays a pivotal role in the physiology of lactation, seems to be an osmoregulatory hormone in fishes [1] and birds [2]. Findings of studies in which the perfused cat kidney [3], the unanesthetized rat [4], the rat with congenital pituitary diabetes insipidus [5] as well as man [6] appear to respond to the administration of prolactin with a decrease in cation or water excretion or both suggest a role for the hormone in the control of fluid balance in mammals as well. That such an action of prolactin may be of physiologic importance, at least in the rat, is further supported by the observed increase in sodium, potassium and water excretion that follows the chronic administration of 2 bromo-alpha-ergokryptine [7], a known inhibitor of prolactin secretion [8]. The effects of increased concentrations of endogenous prolactin on the renal function of man have so far not been fully studied and, although suggested by the above studies, a role of the hormone in the maintenance of water balance remains also to be elucidated. The present study was therefore undertaken to determine *1*) what changes in renal hemodynamics, sodium and water excretion accompany an increase in the blood concentration of endogenous prolactin, and *2*) whether there is an osmolar control of prolactin release in adult man.

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Methods

The study used only healthy male volunteers between the ages of 25 and 50 yr. The employed protocols were approved by the Human Experimentation Committee and all volunteers were fully informed of the nature of the study. All subjects fasted and refrained from smoking from midnight of the day of the study. Experiments were started approximately at 8:00 AM. The effect of prolactin on various indexes of renal function in man was studied in five subjects. These received a priming dose of 50 g of inulin and 2 g of para-aminohippurate (PAH) followed by a sustaining solution of these drugs dissolved in normal saline and administered at 1 ml/min by a roller pump (Holter Co., King of Prussia, PA). A water diuresis was established by the oral intake of 20 ml/kg of water over 30 min. Urine was collected every ten minutes and losses were replaced orally. When the water diuresis was established as evidenced by no further drop in urinary osmolality or an increase in urine flow over three collections, synthetic thyrotopin-releasing hormone (TRH), 7 μg/kg (Abbott Co., North Chicago, IL), a reliable stimulus to endogenous prolactin secretion, was given rapidly intravenously [9]. The activity of the TRH was confirmed by measuring concentrations of TSH (Phadebas TSH test, Pharmacia, Sweden) before and after its administration to five of the subjects. TSH rose in each as the mean levels increased from 2.9 ± 0.4 to 21.7 ± 2.4 U of TSH/ml of serum ($P < 0.001$). After TRH administration, and allowing for a ten-minute equilibration period, urine collections were continued for one hour. Venous blood was drawn without stasis in the fasting state, at the height of the water diuresis just prior to and 20, 40 and 60 min after the administration of TRH. Blood and urine specimens were analyzed for sodium (Flame photometer, Instrumentation Laboratories, Boston, MA), osmolality (Fiske Associates, Uxbridge, MA), inulin [10] and PAH [11]. In addition, blood concentrations of prolactin were measured by radioimmunoassay. Human prolactin (HPRL), composed of isohormones B and C, isolated from amniotic fluid [12], was used for radioiodination and also served as a reference hormone. In contrast to the available pituitary HPRL, this preparation has been found to be stable for one year if kept frozen at $-70°C$, and there has been no difficulty in obtaining a good tracer with it. Rabbit anti-human prolactin was kindly supplied by the Public Health Service, National Institute of Arthritis, Metabolic, and Digestive Diseases, Bethesda, MD; goat anti-rabbit immunoglobulin (Miles-Yeda, Rehovot, Israel) served as the second antibody for the double antibody radioimmunoassay. Iodination with ^{125}I was performed by the method of Greenwood, Hunter and Glover [13]. Separation of the labelled intact protein from damaged molecules and free iodine was carried out in a 22×0.9 cm column containing Sephadex G-75 coated with 0.5 ml of 30% bovine serum albumin (BSA) in phospho saline buffer (PSB) (0.1M, pH 7.6), and the solution was then washed with PSB. The immunoreactive labelled prolactin was found in the second peak, while damaged molecules appeared in the first peak. The specific activity of the labelled prolactin ranged from 80 to 150 mCi/mg. The method was found to be specific for HPRL. Neither human placental lactogen nor human growth hormone was detectable in the assay system. Moreover, large doses of human follicle-stimulating hormone and thyroid-stimulating hormone were completely ineffective in inhibiting the reaction of labelled HPRL with anti-HPRL. To test whether a serum component, other than prolactin, interfered with the inhibition curve of the radioimmunoassay, a "serum standard curve" was also performed; serum with a high prolactin concentration (40 mg/0.1 ml) pooled from women in late pregnancy and hyperprolactinemic patients served as the highest prolactin concentration point on the curve. Also, a standard curve of human pituitary prolactin, obtained from the National Institutes of Health, was constructed. Parallel curves to that of the amniotic fluid HPRL were obtained. The serum with the high prolactin concentration and serum with 1.8 ng/0.1 ml of prolactin, pooled from normal male and female subjects, were used as the "laboratory internal reference" and were run with each assay. The interassay percent variations were found not to exceed 8%. In this laboratory, the concentration of serum prolactin measured in normal adults of either sex at various times of the day is between 4 and 34 ng/ml.

The effect of changes of serum tonicity on prolactin release was studied in six subjects whose serum was rendered hypertonic by a modification of the method of Moses, Streeten and Streeten [14]. Thus, following the establishment of a water diuresis, an infusion of 5% NaCl at 0.05 ml/kg/min was begun and continued for two hours. Upon cessation of the infusion, TRH was administered as described above. Urine was collected every 15 min and blood specimens were drawn in the fasting state, at the height of the water diuresis, at one and two hours after the hypertonic NaCl infusion was begun and 20, 40 and 60 min after TRH administration. Blood and urine specimens were analyzed for sodium and osmolality and blood specimens for prolactin as described above.

The effect of TRH infusion on prolactin release in

Table 1. Effect of thyroid-releasing hormone (TRH) on serum prolactin levels in various states of hydration (ng/ml)[a]

Overnight dehydration			Water-loaded			Hypertonic volume expansion		
Subject No.	Before TRH	After TRH	Subject No.	Before TRH	After TRH	Subject No.	Before TRH	After TRH
1	14	28	1	27	69	1	26	58
2	23	38	2	20	45	2	30	54
3	20	48	6	34	62	6	34	60
4	22	52	7	36	72	7	30	62
5	35	48	8	34	53	9	25	40
						10	21	47
Mean	22.8	42.8		30.2	60.2		27.7	53.5
SEM	3.4	4.3		2.9	5.0		1.9	3.5
P value	<0.01			<0.005			<0.001	

[a] Prolactin concentrations are 20 min after TRH administration.

the hydropenic state was studied in five subjects following overnight dehydration. Prolactin concentrations were determined 20 min before, just prior to and 20, 40 and 60 min after TRH administration. Student's *t* test was employed for statistical analysis. A *P* value greater than 0.05 was considered not significant.

Results

Effect of TRH on prolactin release in hydropenic man. The i.v. administration of 7 μg/kg of TRH caused no changes in systemic pressure, which was measured in approximately 50% of the subjects. Most of them did report a sensation of nausea which subsided within minutes. The ability of the tripeptide to cause a consistent and significant increase in serum prolactin when given after an overnight fast is shown in Table 1, left panel. The magnitude of increase is similar to that reported previously [9] as mean concentration rose from 22.8 ± 3.4 to 42.8 ± 4.3 ng/ml ($P < 0.01$). The peak response was uniformly recorded at 20 min, and it is this value that is reported throughout this study.

Effect of prolactin on renal hemodynamics, sodium and water excretion. As noted in the middle panel of Table 1, water loading by no means altered the prolactin stimulatory effect of TRH. Thus, the level of prolactin rose well above the physiologic range in all five subjects as mean concentrations increased from 30.2 ± 2.9 to 60.2 ± 5.0 ng/ml ($P < 0.005$). As shown in Table 2, this increase in serum prolactin levels was associated with no alteration in water excretion in any of these subjects as both free water clearance and urinary osmolality were unchanged. Likewise, no statistically significant change in either inulin clearance, PAH clearance or the rate of sodium excretion was observed in association with the increased concentrations of prolactin.

Effect of alterations in serum tonicity on prolactin release. A decrease in serum tonicity was achieved by an oral water load 11 times in seven subjects (Table 3). The load caused the serum sodium concentration to decrease from 141 ± 1.0 to 137 ± 1.0 mEq/liter ($P < 0.001$) as serum osmolality decreased from 293 ± 1.7 to 285 ± 1.5 ($P < 0.001$). The serum prolactin concentration measured at the peak of the water diur-

Table 2. Effect of increased prolactin levels on renal hemodynamics, sodium and water excretion[a]

Subject No.	Inulin clearance *ml/min*		PAH clearance *ml/min*		Sodium excretion *μEq/min*		Free water clearance *ml/min*		Urinary osmolality *mOsm/kg H_2O*	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
1	128	118	537	497	188	177	11.1	10.4	67	69
6	118	134	791	752	216	272	14.4	16.8	58	56
8	135	134	760	786	190	299	16.1	19.1	49	56
2	98	86	548	451	67	82	9.5	9.8	80	85
7	112	100	551	503	202	247	13.2	12.9	71	63
Mean	118.2	114.4	637	597	173	215	12.9	13.8	65	66
SEM	6.4	9.5	57	71	27	39	1.2	1.8	5.3	5.4
P value	NS		NS		NS		NS		NS	

[a] Each value is the mean of three to four collections. Those in the experimental period are means of collections taken 15 to 60 min after TRH administration.

Table 3. Effect of water loading on serum sodium, serum osmolality and serum prolactin in man

	Serum sodium *mEq/liter*		Serum osmolality *mOsm/kg*		Serum prolactin *ng/ml*	
	Control	Water load	Control	Water load	Control	Water load
6	144	141	290	283	32	34
6	139	134	286	280	30	36
1	140	134	287	281	27	27
1	146	143	293	284	25	36
7	138	133	288	277	35	38
7	138	135	292	287	33	36
2	139	136	303	293	15	20
2	143	138	302	290	26	21
8	142	139	292	285	35	39
9	140	138	297	292	25	20
10	137	134	295	287	23	22
Mean	141	137	293	285	28	30
SEM	1.0	1.0	1.7	1.5	1.8	2.4
P value	<0.001		<0.001		NS	

esis (90 to 120 min after the beginning of the water load) was not significantly altered from control, 28 ± 1.8 to 30 ± 2.4 ng/ml.

Serum hypertonicity was achieved by the infusion of 5% NaCl in six subjects undergoing a water diuresis (Table 4). The infusion was associated with an increase in serum sodium from 137 ± 1.4 to 145 ± 1.6 mEq/liter ($P < 0.001$) as serum osmolality rose from 287 ± 1.8 to 298 ± 1.4 ($P < 0.001$). Each subject responded, as expected with a marked antidiuresis, as mean urinary osmolality rose from 62 ± 5.9 to 480 ± 48 ($P < 0.001$). These marked changes in serum tonicity and renal water excretion were associated with no consistent alteration in serum prolactin concentration as the mean remained essentially unchanged at 28 ng/ml. The levels of the hormone at one and two hours after initiation of the hypertonic NaCl infusion were very similar, and the values at two hours are shown in the table for all indexes. Furthermore, the hypertonicity and volume expansion neither blunted nor enhanced the subject's response to TRH as shown in Table 1, right panel. As was the case in the previous groups, here too baseline prolactin concentrations almost doubled as the mean increased from 27.7 ± 1.9 to 53.5 ± 3.5 ng/ml ($P < 0.001$).

Discussion

Considerable evidence has accumulated to suggest that the anterior pituitary hormone prolactin plays a role in the adaptation of some fish to a fresh water environment by altering the handling of sodium and water in these animals [1]. An effect of the hormone on the mammalian kidney was demonstrated by Lockett and Nail who showed that sheep prolactin alters renal hemodynamics and water excretion in the cat and the rat [3, 4]. The administration of prolactin to the rat with congenital diabetes insipidus is also associated with an antidiuresis, suggesting a vasopressin-independent mechanism [5]. However, in this latter study the increase in urinary osmolality was modest and the failure of the urine to attain even the level of serum osmolality places considerable doubt on the ability of prolactin to significantly increase the water permeability of the collecting duct epithelium

Table 4. Effect of hypertonic NaCl administration on serum sodium, serum osmolality, urinary osmolality and serum prolactin in man

	Serum sodium *mEq/liter*		Serum osmolality *mOsm/kg H_2O*		Urinary osmolality *mOsm/kg H_2O*		Serum prolactin *ng/ml*	
	Control	Hypertonic saline	Control	Hypertonic saline	Control	Hypertonic saline	Control	Hypertonic saline
1	143	149	290	299	58	415	36	26
2	138	150	284	301	77	651	20	30
6	134	140	280	292	51	412	36	34
7	135	144	287	297	45	372	36	30
9	138	143	292	301	61	422	20	25
10	136	141	287	297	82	607	22	21
Mean	137	145	287	298	62	480	28	28
SEM	1.4	1.6	1.8	1.4	5.9	48	3.4	1.9
P value	<0.001		<0.001		<0.001		NS	

and thus suggests a different mechanism for the observed antidiuresis.

In contrast to a number of other hormones [15], the effect of prolactin on water excretion in man has received only scant attention. Since prolactin preparations are frequently contaminated with antidiuretic hormone (Cronin R, Anderson R: unpublished observation), in the present study we chose rather to increase the levels of endogenous prolactin by the i.v. injection of TRH. Although this tripeptide itself could conceivably alter systemic hemodynamics or cause other hormonal changes that could alter sodium and water excretion, no such effects have so far been described *in vivo*. The increase in endogenous prolactin concentration had no effect whatsoever on the renal excretion of water. The clearance of PAH decreased in four of the five subjects studied, but in view of the variability in this determination, this change was not statistically significant. A decrease in renal blood flow, however, would in itself tend to decrease urine flow and free water clearance and thus would have tended to potentiate rather than obscure an antidiuretic effect of prolactin. In spite of the tendency of the clearance of PAH to decrease, the excretion of sodium rose slightly in four of the subjects, an increase that again did not reach statistical significance and that could hardly have obscured an antidiuretic response. Our results failing to show an effect of increased prolactin concentrations on water excretion and, if anything, a small tendency to increase sodium excretion is thus in conflict with the findings of Horrobin et al [6], who reported a decrease in urine flow following the i.m. administration of 8 mg of sheep prolactin. It is unclear from their study, however, whether the decrease in urine flow was due to a decrease in free water clearance or primarily a consequence of the decreased solute clearance that must have occured as sodium and potassium excretion also declined. This latter effect on cation excretion could have been due to the growth hormone, which the authors acknowledge as a contaminant, since growth hormone has been noted to have sodium-retaining properties itself [16]. Although the concentrations of TSH and thyroid hormone must also have increased following TRH administration, it seems most unlikely that these would entirely obscure or antagonize a possible antidiuretic effect of prolactin. In fact, the observed effect of TSH on salt and water transport in the toad bladder [17] if applicable *in vivo* at the levels attained in this study should have itself caused an antidiuresis. Since the concentrations of prolactin reached in our study—although clearly above the normal range—are not extremely high, we cannot exclude the possibility that much higher levels of the hormone as have been measured in patients with pituitary tumors [18] or more chronic stimulation with the hormone may affect renal water handling by either proximal or distal tubular mechanisms. This could explain the results in the above study [6] in which, even assuming a very large volume of distribution, very high concentrations of prolactin must have been reached, as well as the recent preliminary description of an abnormality in water excretion in patients with hyperprolactinemia [19]. The failure of any of our water diuresing subjects to respond to an increase in endogenous prolactin with an antidiuresis allows us to conclude that in the achieved concentrations the hormone has no appreciable effect on the water permeability of the collecting duct nor on fluid reabsorption in the proximal tubule since such changes would also be expected to change urine flow in the vasopressin-free state. The results do not exclude the possibility that the hormone could affect water excretion in the presence of vasopressin or affect sodium and water movement in the proximal tubule in a different state of sodium balance.

Were prolactin to play a physiologic role in the control of water balance in man, it would be expected that the state of hydration be a determinant of the hormone's release. Since there is considerable controversy on this issue in the literature, with earlier studies claiming the existence of an osmoregulation of the hormone's release [18, 20], but a more recent one finding no evidence for it [21], we undertook to measure the concentrations of prolactin in hypotonic and hypertonic states. Our results reveal that neither an acute decrease in serum tonicity as achieved with an oral water load nor an acute increase in serum tonicity as achieved with 5% NaCl significantly altered the measured concentrations of prolactin in our subjects. In fact, the linear correlation coefficient between simultaneously measured serum sodium and prolactin was 0.176 and between serum osmolality and prolactin 0.341, neither of which are significant. Our results are therefore in disagreement with those of Buckman et al, who found that water loading decreased prolactin concentrations [18, 20]. Our sample taken at the height of the water diuresis, 90 to 120 min after the water load was begun, closely corresponded to the time at which the authors encountered the lowest prolactin concentrations. The authors also conclude in the latter study [20] that hypertonicity causes the release of prolactin. Further scrutiny of their data reveals that the four subjects who received hypertonic NaCl over 30 min attained serum osmolalities comparable to those achieved in the present study. The serum prolactin concentration, how-

ever, was increased in only two of them by the end of the infusion period; 20 min later the prolactin concentration had returned to control levels in one of them but had become elevated in a third subject. In our study none of the six subjects rendered hyperosmotic significantly increased their serum prolactin concentration although they were clearly capable of so doing as shown by the subsequent response to TRH. Our results, therefore, are in agreement with the recent report of Adler et al [21], who likewise failed to observe changes in serum prolactin concentration in association with alterations in serum tonicity. Taken together, therefore, our data provide no evidence for an osmoregulation of prolactin release in man or for an effect of supraphysiologic levels of the hormone on renal water excretion in the vasopressin-free state. A role of prolactin, if any, in the control of fluid balance in states in which its levels rise such as the postpartum period [22] or sleep [23], in which the levels of hormone measured are somewhat lower than those achieved in this study, is at this time speculative and will require further investigation.

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Reprint requests to Dr. Tomas Berl, Department of Medicine, University of Colorado Medical Center, 4200 E. Ninth Ave., Denver, Colorado 80220, U.S.A.

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